MECHANICAL TISSUE RESUSCITATION TREATMENT REDUCES BRAIN TISSUE VOLUME AND INTRACEREBRAL HEMORRHAGE AND INCREASES BLOOD PERFUSION IN A TRAUMATIC BRAIN INJURY MODEL IN SWINE

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ABSTRACT

Each major war tends to have a 'signature injury', with traumatic brain injury (TBI) associated with the Iraq war (Operation Iraqi Freedom II and Operation Enduring Freedom) due to the high incidence of personnel injured by IED (improvised explosive devices). Based upon successful outcomes in a rat model, this study in swine examined the efficacy of application of either 50 or 100 mm Hg vacuum to a controlled cortical impact (focal injury). Based on MRI and histological analysis, 100 mm Hg applied immediately after injury and continued for 72 hours was more efficacious than 50 mm Hg in decreasing the volume of injured tissue and the volume of hemorrhage.

1. INTRODUCTION

A greater percentage of military personnel are surviving injuries that were fatal in previous wars due to better protective equipment, improvements in polytrauma care, and more expeditious transport to facilities capable of providing higher levels of care.(Bagg, 2006; Gawande, 2005) Each major war tends to have a 'signature injury', with traumatic brain injury (TBI) associated with the Iraq war (Operation Iraqi Freedom II and Operation Enduring Freedom) due to the high incidence of personnel injured by IED (improvised explosive devices).(Colombo, 2008; Galarneau, 2008; Ritenour,2008; Warden, 2006) For injured personnel evacuated to Walter Reed who sustained injuries from hostile forces, 28% had TBI with the percentage rising to 50% of patients in the ICU.(Colombo, 2008; Warden 2006)

While obvious, the brain is encased in a bony cavity which limits its ability to swell. Following injury, the volume of the tissue can not increase so the pressure within the brain increases, similar to other compartment syndromes. The pressure quickly rises above local blood pressure, causing ischemia and decreasing the amount of available oxygen. The decrease in oxygen is associated with decreased outcomes.(Bardt, 1998; Chesnut, 1993; Ritenour, 2008; van den Brink, 2000) Two types of brain edema are present: vasogenic edema in which the blood brain barrier (BBB) is damaged by mechanical injury or autodestructive mediators (or both) and protein rich exudate derived from plasma shifts from the vasculature into the tissue; and cytotoxic brain edema which is characterized by intracellular water accumulation.(Menon, 1999; Untenberg, 2004) Edema due to secondary injury progresses over 24 to 72 hours.(Kawamata, 2007) This progression of secondary injury is responsible for the "talk and die" phenomena in which patients with apparent mild TBI progressively decline and either die or lapse into a permanent vegetative state.(Davis, 2007)

A wide variety of pharmacologic interventions have been proposed, with some in clinical trials, but none have proven to be successful enough to be routinely implemented.(Bullock, 1995; McIntosh, 1996; McKeating, 1998; Morgan-Kossmann, 2001). A potential problem with pharmacologic interventions is that several cascades are initiated, and trying to block a single point among the several pathways is not effective. As a non-specific surgical alternative, decompressive craniectomy is routinely performed on patients with traumatic brain injury with increased intracranial pressure that is non-refractory to medical measures. While it does not address any specific factor, removal of a window from the skull allows for the brain tissue to expand outside of the cranial vault and decreases pressures. In a prospective, randomized trial it has been associated with good long term results – not just life or death, but also quality of life.(Timofeev, 2006)

Application of sub-atmospheric pressure (The V.A.C.™ :KCI, San Antonio, TX) has been extremely successful in treating a wide variety of soft tissue injuries and conditions of increased pressure system. It currently is used in the Iraq theater for treatment of soft tissue wounds, particularly those of the extremities.(Covey, 2006; Geiger, 2008; Leininger, 2006) It is also used to successfully treat injuries associated with high energy trauma.(Dedmon, 2007; Stannard, 2006) Due to the differential in pressure between the vacuum dressing and the tissues, fluid flows from the tissue into the device, decreasing the intrastitial pressure and volume and allowing for successful treatment and closure of wounds associated with compartment syndromes (both of the

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14. ABSTRACT
Each major war tends to have a ?signature injury‟, with traumatic brain injury (TBI) associated with the Iraq war (Operation Iraqi Freedom II and Operation Enduring Freedom) due to the high incidence of personnel injured by IED (improvised explosive devices). Based upon successful outcomes in a rat model, this study in swine examined the efficacy of application of either 50 or 100 mm Hg vacuum to a controlled cortical impact (focal injury). Based on MRI and histological analysis, 100 mm Hg applied immediately after injury and continued for 72 hours was more efficacious than 50 mm Hg in decreasing the volume of injured tissue and the volume of hemorrhage.

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extremities and abdomen). (Lee, 2005; Perez, 2007; Yang, 2006) Within the fluid that is removed are soluble factors associated with inflammation and healing. (Moues, 2008; Stechmiller, 2006) The removed fluid may also contain non-native factors detrimental to healing including toxins, venom and chemotherapeutic agents. (Morykwas, 1999a; Van Gossler, 2000) We have shown that application of sub-atmospheric pressure to crush injuries removes myoglobin in the fluid, preventing its entry into the systemic circulation and preventing its eventual damage to the kidneys. (Morykwas, 2002)

In traumatic, focal brain injuries there is the central area of necrosis (death due to trauma) which is surrounded by a 'halo' of damaged tissue which progressively dies due to sequela of the above mentioned cascades. This is similar to burn injuries, in which there is the zone of coagulation (death due to thermal injury), surrounded by the zone of stasis (tissue progressively dies due to ischemia, re-perfusion injury, infection, etc.) In a swine model of partial thickness burn injury, application of sub-atmospheric pressure was able to successfully save the tissues in the zone of stasis – tissues that in the burns treated with the standard of care did progressively die. (Morykwas, 1999b)

Combining the results of applying sub-atmospheric pressure to successfully removing soluble factors and toxins with the ability to interrupt the cascades with result in death of cells in the zone of stasis in burn injuries was the impetus to apply sub-atmospheric pressure to a cortical injury in a rat model. In our preliminary study examining the treatment of controlled cortical impact injuries to rat brains, application of sub-atmospheric pressure was successful in significantly decreasing water content and volume of the injured area, and also significantly decreasing levels of excitatory amino acids and lactate in treated animals compared to non-sub-atmospheric pressure treated animals. (Argenta, 2008) These results led to the current study in which sub-atmospheric pressure is applied to a focal injury on the gyrencephalic brain of swine.

2. MATERIALS AND METHODS

2.1 Animal Model

Twenty one female domestic pigs (22-33 kg) were procured and randomly divided into three groups: operated sham (n=3); controlled cortical impact (CCI) non-treated (n=7); CCI MTR 50 mm Hg treated (n=5); or CCI MTR 100 mm Hg treated (n=6). For creation of the CCI, animals were anesthetized and a 17 mm diameter craniotomy was performed over the right front parietal cortex. A pneumatic impactor pistol was used with the plunger parameters of 15 mm diameter, 12 mm in depth, 2.7m/s velocity, and 250ms dwell time. For MTR treatment, a sterile vacuum dressing was placed in the bony defect and either 50 mm Hg or 100 mm Hg vacuum was applied continuously for 72 hours. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Wake Forest University.

2.2 Intracranial Pressure Monitoring

A 2 mm diameter hole on the contralateral side was drilled through the cranium 4mm lateral to midline and 4 mm posterior to the bregma. Intracranial pressures (ICP) were monitored by inserting PA-C40 pressure probe around 2 cm in depth (cranial bone thickness is 4-6 mm) and recorded by DSI telemetry units (Data Sciences International; St. Paul, MN). The ICP data were collected every 30 min without interferences such as the animal running or jumping. Total numbers of animals measured: Injured (n=5); Injured + MTR 100 mm Hg (n=6); Injured + MTR 50 mm Hg (n=5); and sham surgery (n=3).

2.3 MRI Procedures

MRI was performed with a GE (Milwaukee, WI) Signa EchoSpeed 1.5-T scanner. Animals were maintained under isoflurane anesthesia and placed in 8-channel HR Brain coil. Localizer scans were run. Parameters analyzed included: MR sagittal T1 imaging; coronal T2 imaging; coronal MPGR (Multi-Planer Gradient Echo); Axial T2* Contrast Enhanced Perfusion (0.2 ml/Kg Magnevist contrast by power injection). Anatomic images were collected using the routine head protocol for coronal T2 enhanced fast spin-echo (FSE-XL, TE 84.82 msec, TR 8000 msec, slice thickness/gap 2/0 mm, field of view [FOV] 13 cm, matrix 128 × 128, number of excitations [NEX] 4, Scan time was 6 minutes). T2*-weighted gradient-echo MR imaging is useful in the detection of old intracerebral hemorrhage. Coronal MPGR (Multi-Planar Gradient Echo, TR 616 msec, TE 11 msec, flip angle 15 degrees, thickness/gap 2 / 0 mm, FOV 13x13 cm, matrix 128 x 128, NEX 2, scan time was 5 min 23 sec) was performed.

All MRI measurements were performed on TeraRecon workstation. Total contusion injured brain volumes were measured in all coronal MR T2 weighted images as the sum of all injury areas in both groups. The injured area was identified and traced as a hyperintense region ipsilateral to the injured site. There was a large area of T2 hyperintensity (edema) sometimes associated with hypointensity (hemorrhage) and herniation in T2-weighted images. Totals analyzed: Injured only (n= 7); 50 mm Hg treated (n=5); 100 mm Hg treated (n=7).

2.4 Histology

All animals were euthanized and perfused with 4% paraformaldehyde through the ascending aorta 9 days post-injury. The brain was removed and postfixed in the same fixative solution overnight at 4ºC. After rinsing in PBS, the brains were placed in 30% sucrose at 4°C before they were snap-frozen in O.C.T. (Sakura Finetek USA, Inc. Torrance, CA). The brains were kept at -80ºC until use. Coronal sections of the injured area were cut into 50 μm in thickness using a cryostat (Leica, Germany), mounted, and kept frozen until use. Sections were collected every 1.6 mm through the entire injured area over 1.76 cm. Sections were examined after staining with haematoxylin and eosin (H&E). Areas of necrosis, hemorrhage, and non-artifact cavities were used to estimate volume by approximating slices through an ellipsoid. Totals analyzed: Injured only (n= 4); and 100 mm Hg treated (n=4). One animal treated with 50 mm Hg was analyzed due to perfusion problems.

3. RESULTS

3.1 Contusion Volume by MR T2 Weighted Images

Total contusion injured brain volumes were measured in all coronal MR T2 weighted images as the sum of all injury areas in all three groups groups. The injured area was identified and traced as a hyperintense region ipsilateral to the injured site. There was a large area of T2 hyperintensity (edema) sometimes associated with hypointensity (hemorrhage) and herniation in T2-weighted images. (Figure 1)

Three days after CCI, the mean contused brain tissue volume was 3.44 $\pm$ 1.14 cm^3 for the MTR 100 mm Hg treated animals. This is significantly ($p < 0.01$) smaller than with the volume for the injured only animals (6.59 $\pm$ 1.76 cm^3)in non-treated injured animals and the animals treated with 55 mm Hg (9.49 $\pm$ 3.71 cm^3). The difference between the injured only and the 50 mm Hg treated animals was not significantly different. (Fig. 2)

3.2 Injured Tissue Volume by Histological Analysis

At 9 days after injury, histopathologic results demonstrated major neuronal tissue loss and intracerebral hemorrhage in non-treated injured brains (Figure 3 left), which confirmed that hypointense lesions seen on T2-weighted and gradient echo MR images were hemosiderin deposits of hemorrhages after injury. Less neuronal loss and hemorrhage in the injured area were observed after 100 mm Hg MTR treatment (Figure 3 right). Mean estimated volume of death and damage for 100 mm Hg treated animals was 387.8 + 205.1 mm^3. Mean estimated volume of death and damage for injured, non-treated animals was 808.75 + 361.7 mm^3. The estimated volume of death and damage for the 50 mm Hg treated animal was 728.73 mm^3.

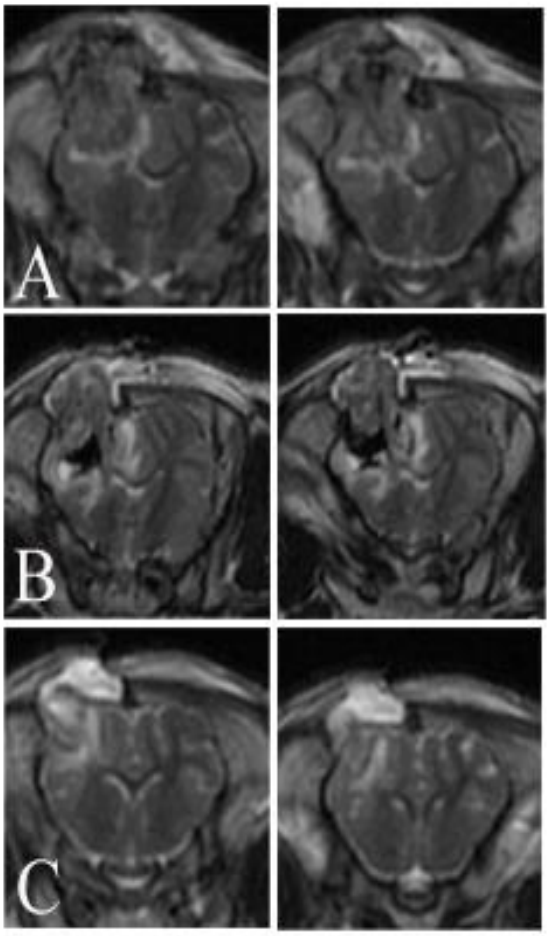

Fig 1. Representative T2-weighted MR images of pig brain Injury with/out MTR treatments. Brain tissue injured volume was measured in each coronal T2 weighed MRI. A is injured only, B is injured pig treated with 50 mmHg MTR and C is injured pig treated with 100 mmHg MTR.

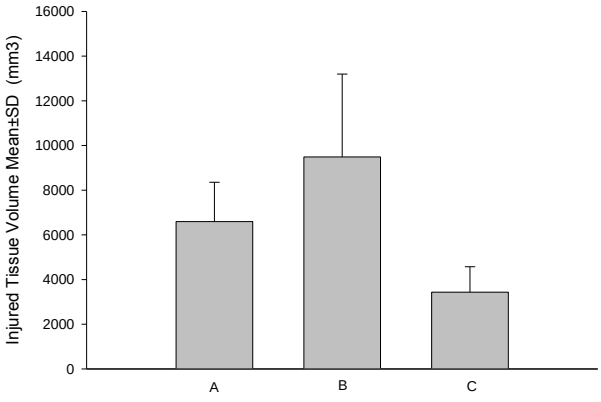

Figure 2. The mean total brain tissue injury volumes measured in T2-weighted MR images. A is injured only, B is injured with 50 mmHg MTR and C is injured pig treated with 100 mmHg MTR.

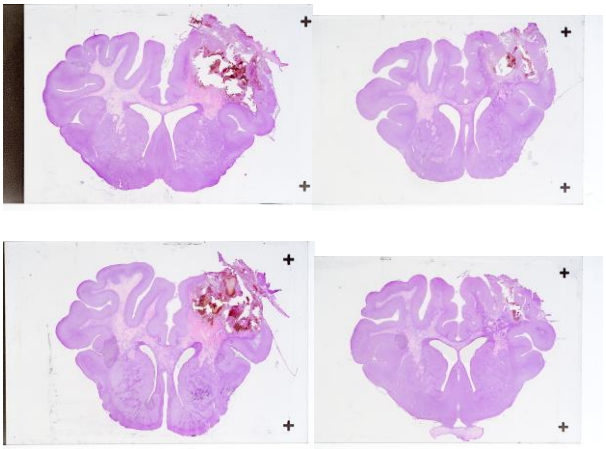

Figure 3. Left. Injured, non-treated brain slices 9 days post injury. Right. Injured, MTR (100 mm Hg, 72 hour treatment) treated brain slices 9 days post injury. Slices are 3 mm apart through the center of CCI site. H&E. Original magnification 2X.

3.3 Intracerebral Hemmorhage Volume

The total intracerebral hemorrhage volume was measured in all positive coronal images of MRI gradient echo. (Figures 4 and 5). The mean hemorrhage volume in injured only, non-treated animals (N=7) is 375.75±348.9mm^3. The mean hemorrhage volume in animals treated with MTR 50 mmHg is 606.84±364.05 mm^3. The mean hemorrhage volume in injured animals with MTR 100mmHg treatment (C) is 53.31±67.81 mm^3 (N=6). The mean volume for the animals treated with 100 mm Hg is significantly ($p < 0.01$) smaller than untreated and those treated with 50 mm Hg. There is no statistical difference comparing injured only animals to those in the 50 mm Hg group.

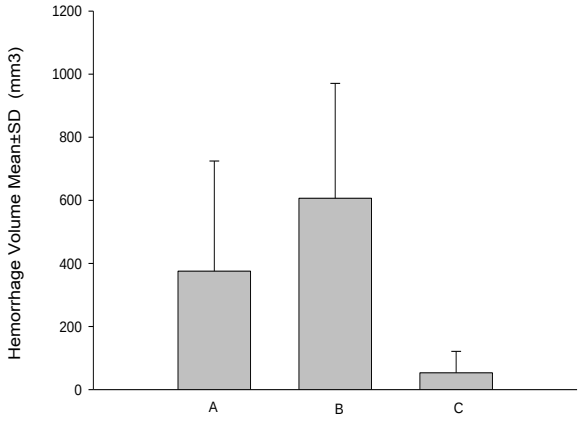

Figure 4. Volume of hemorrhage in (A) injured, non-treated animals, (B) animals treated with 50 mm Hg, and (C) animals treated with 100 mm Hg for 72 hours.

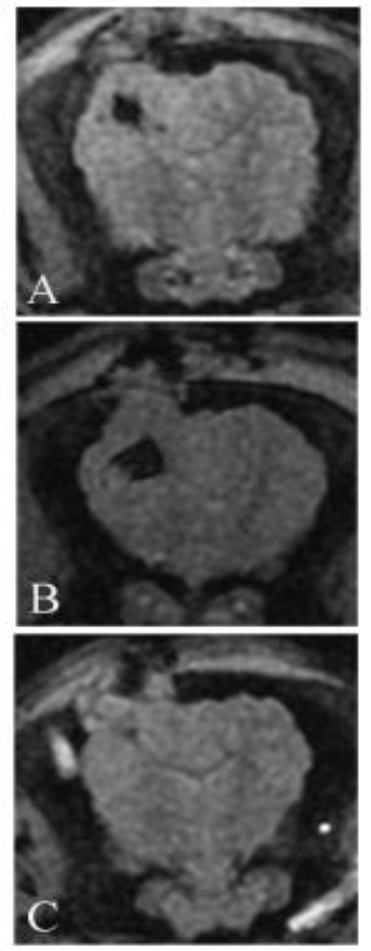

Figure 5. Representative gradient echo MR images of pig brain Injury with/out MTR treatments. Brain tissue injured volume was measured in each coronal T2 weighed MRI. A is injured only, B is injured animal treated with 50 mmHg MTR, and C is injured animal treated with 100 mmHg MTR for 72 hours.

3.4 Intracranial Pressure

The intracranial pressure for those animals treated with 100 mm Hg was consistently lower than for the operated sham animals, injured only, and also those treated with 50mm Hg. Animals treated with 50 mm Hg consistently exhibited higher intracranial pressures than all other groups. (Figure 6)

4. DISCUSSION

Traumatic brain injuries consist of both primary injury, tissue that is damaged or killed from the insult, and secondary injury, cells and tissue that progressively die due to pathophysiological cascades resulting in increased cerebral edema and decreased cerebral blood flow. These cascades include the release of excitatory

(EAA) and toxic amino acids, focal and global ischemia with concomitant increases in lactate, and changes in water content – all of which may lead to delayed or chronic neuronal death.

Cell death following traumatic brain injury is biphasic, with initial, primary death due to the trauma itself, then an ongoing, secondary apoptotic or necrotic death as sequela to the release of excitatory amino acids, buildup of lactate, etc.(McIntosh, 1996; Raghupathi, 2004) (The release of excitatory amino acids (glutamate, aspartate) cause a disturbance in ion homeostasis via agonist opened channel, thus increasing energy demand and increasing lactate production.(Allessandri, 1999; Bullock, 1995) Microdialysis studies of human patients who have suffered a traumatic brain injury show very high levels (up to 50 X normal) in patients with focal contusions and in patients with secondary ischemic events, with elevated levels lasting up to four days post injury.(Zauner, 1997; Zoremba, 2007) Elevated levels of glutamate have been shown to be correlated with increased levels of lactate. This increase in lactate is reflective of increased energy demand during periods of impaired supply (ischemia), and is inversely related to patient outcome. (Zauner, 1997) Lactate production leads to apoptotic neuronal cell death.(14, 16) Relevant to this study, lactate increases following controlled cortical impact, both in rats and swine. (Alessandri, 2003; Thomale, 2007)

Initiation of a variety of cascades result in the release of excitatory amino acids, ions shifts, release of proteases, oxygen radicals, complement proteins and other immune mediators cause concomitant activation of the neuroinflammation cascade. (Morganti-Kossmann, 2001) Disruption of the blood brain barrier (BBB) allows for transfer of intravascular proteins to the interstitium and for migration of neutrophils into the brain tissue. (Morganti-Kossmann, 2001) This results in inflammation at the injured site with release of inflammatory mediators including cytokines and adhesion molecules. (McKeating, 1997; McKeating, 1998; Morganti-Kossmann, 2001; 26-31)

Related to a non-specific treatment, the major thrust of this study is to apply controlled, localized sub-atmospheric pressure (vacuum or 'negative pressure') to the craniotomy site, decreasing edema and removing soluble mediators. As shown by the initial results, application of 100 mm Hg sub-atmospheric pressure to the site of injury decreased both the volume of injured tissue and the volume of hemorrhage following injury. It is not known why the animals treated with 50 mm Hg exhibited a greater volume of death and hemorrhage than the control, non-treated animals.

Additional analysis of MRI data currently being perfomed is perfusion and MR spectroscopy data. Complimentary computer programs are being developed to allow for expansion of human analysis to a porcine brain for perfusion and MR spectroscopy analysis. Baseline scans in un-injured animals have been collected and all scans await processing to determine the effects of application of sub-atmospheric on both perfusion and metabolic changes in the injured area. Current studies are determining the length of time the sub-atmospheric pressure needs to be applied, and also the delay between injury creation and the efficacious application of sub-atmospheric pressure.

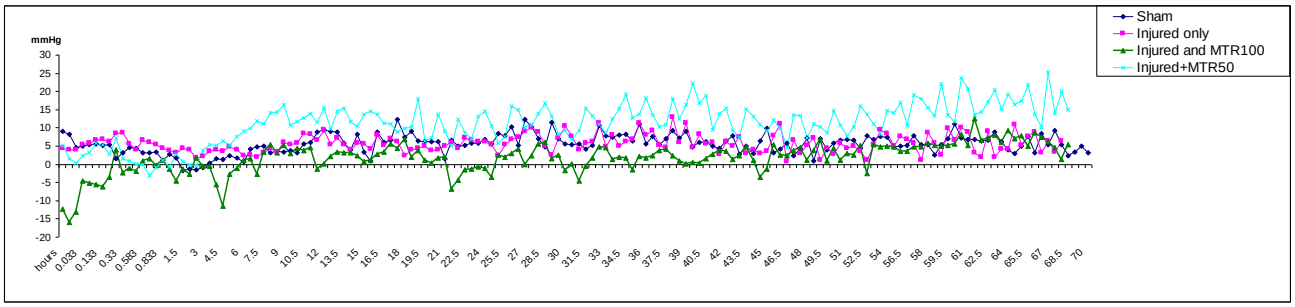

Figure 6. The mean intracranial pressures (ICP) monitoring in swine following a traumatic brain injury with and without 50 or 100 mm Hg MTR treatment for 3 days post injury. The ICP was recorded every 30 minutes in each animal. X axis = hours (up to 72). Y axis is intra cranial pressure in mm Hg.

5. CONCLUSIONS

This study demonstrates that the use of mechanical tissue resuscitation (MTR) treatment reduces the extent of brain tissue injury when applied immediately post injury. MTR treated animals demonstrated a decrease in intracerebral hemorrhage and neuronal tissue loss. Further studies are required to determine efficacy of MTR with increasing times between CCI injury and application of MTR. If MTR shows similar efficacy is conserving brain tissue following a reasonable delay in application, the technique will be a major advancement in the treatment of personnel with TBI.

The decrease in severity of injury may allow for return of combat personnel to active duty following relatively minor injury, or may decrease the severity of impairment following more significant injuries.

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